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Chitosan–pDNA nanoparticle characteristics determine the transfection efficacy of gene delivery to human mesenchymal stem cells

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Abstract

Purpose: **This study evaluated the potential for prepared chitosan–plasmid DNA (pDNA) nanoparticles to transfer an exogenous gene into human bone marrow-derived mesenchymal stem cells (MSCs).** ***Methods:*** **Chitosan–pDNA nanoparticles were synthesized by the complex coacervation method. We used 18, 50 and 136 KD chitosan at concentrations of 0.05%, 0.1%, 0.5% and 1%, in addition to a pTracer-CMV2 plasmid that contained the green fluorescent protein (GFP) gene. To examine the complexation, samples were run through an agarose gel. The sizes and zeta potential of the nanoparticles were measured by a nanosizer. Scanning electron microscopy (SEM) imaging was used to observe the nanoparticle morphology. MSCs were prepared from human bone marrow and transfected with chitosan–pDNA nanoparticles. The cultures transfected by lipofectamine^{2000} were taken as the control. Cell viability was determined by MTT assay and transfection efficiency by flow cytometry.** ***Results:*** **The smallest size of complexes was obtained with 18 KD chitosan (211 nm) and the highest zeta potential was observed with 136 KD chitosan (29.61 mV). The best transfection rate (18.43%) was achieved with the 0.1% concentration of 18 KD chitosan nanoparticles versus 40.57% for commercial lipofectamine ($p < 0.01$). The MTT assay indicated an average of 95.5% cell viability for 0.1% concentration of 18 KD chitosan compared with approximately 60% of Lipofectamine^{2000}.** ***Conclusion:*** **Nanoparticles produced by 18 KD chitosan at the 0.1% concentration and pDNA may be a promising gene delivery system for human marrow-derived MSCs. Although transfection efficiency of such nanoparticles is lower than that of Lipofectamine^{2000}, however comparatively they possess less cytotoxic effects.**

Keywords: chitosan, gene transfection, mesenchymal stem cells, nanoparticle, plasmid DNA

Introduction

With the advancement of genomic and proteomic technologies, the prospect for gene therapy has progressed rapidly. Gene therapy is the treatment of human disorders by introduction of genetic material into specific target cells of a patient, where an encoded protein will be produced (Corsi et al. 2003). Viral and non-viral vectors are the main systems that can be used to transfer foreign genetic material into a specific target cell (Quong and Neufeld 1998). Viruses such as retrovirus, herpes simplex virus, lentivirus, adenovirus and adeno-associated virus are the most common viral vectors in use (Oligino et al. 2000). In contrast to their high transfection efficiency compared with non-viral vectors (Quong and Neufeld 1998), clinical application of viral vectors is limited due to their immunogenicity, potential for infectivity, complicated production and inflammation (Smith 1995). Naked DNA is one of the simplest nonviral gene delivery systems, however only 1–3% of the cells in different target tissues take up this DNA, resulting in low production of the encoded protein (Mansouri et al. 2004). Cationic phospholipids and cationic polymers are two major types of non-viral gene delivery systems. Both types interact electrostatically with negatively charged DNA and produce complexes (lipo- or polyplexes). Low immunogenicity, ease of preparation, targetability and stability in storage are major advantages of cationic phospholipids and cationic polymers over viral vectors (Deshpande et al. 1998). Polyplexes possess low cytotoxicity and are more stable compared with lipoplexes (Leong et al. 1998).

Chitosan is a cationic polymer that would be a good gene carrier candidate due to its biocompatibility, biodegradability and cationic potential. In addition, chitosan can protect DNA against nuclease degradation. The chitosan molecule is

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